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An Investigation into the Mechanistic Interplay between Th1 and Th2 Cells in Allergic Disease

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Introduction

The incidence of allergic disease has increased steadily in western countries over the past few decades (7,20). Substantial amounts of research have been geared toward elucidating the mechanisms whereby some people develop allergies and others do not. As with all development theories, the question revolves around the debate of genetics versus environment – attempting to determine the relative contribution from heredity and nurture in the development of an allergic phenotype.

Heredity has long been identified as a risk factor for allergies (15). Children born of atopic (allergic disease attributed to heredity) parents are at an increased risk for developing atopy themselves (15). Consequently, genetics is obviously a component of the larger picture. However, rates and symptoms of allergy vary in prevalence both within and between countries inhabited by similar ethnic groups, suggesting that environment and lifestyle choices must play a part as well (36). The International Study of Asthma and Allergies in Childhood was founded in the last couple of years to establish a standardized methodology to investigate both prevalence and cause of allergies worldwide (7). This indicates that concern has risen enough over the possibility of an increased prevalence of allergic disease to warrant a systematic worldwide investigation to span a number of years. Developing countries have not seen the same increase in allergic diseases as the more "westernized" countries, leading to the hypothesis that some aspect of the western lifestyle, such as smaller family size, altered diet, or decreased exposure to infectious diseases must account for this change (20).

At the cellular and molecular level, the mechanism widely believed to control the development of the allergic phenotype is the antagonism between the helper T cell subsets, Th1 and Th2 (35). Helper T (Th) cells mediate their effects on the immune system primarily through cytokine secretion, and the issue of cytokine production is a central component of the

mechanistic analysis of Th1 and Th2 cells. Cytokines are small molecular weight proteins that are involved in a complex series of regulatory pathways that control the differentiation of the different helper T cell subsets and the level of cell proliferation (5). Through their T cell receptor (TCR), naïve helper T cells form a tertiary structure with the major histocompatibility complex (MHC)-peptide structure on an antigen presenting cell (APC) and then either through autocrine (the secreting and responding cell is the same) or paracrine (the secreting and responding cells are different) cytokine signaling, the Th cell is activated and differentiates. The type of cytokine received determines the differentiation (12,28). The cytokines driving Th1 and Th2 differentiation are interleukin-12 (IL-12) (12) and IL-4 (28) respectively. Each Th subset is characterized by a different cytokine profile and effector functions.

Th1 cells are highly protective against infections mounted by many, especially intracellular, microbes due to their ability to secrete Th1 cytokines to activate phagocytic and cytotoxic cells, which are cells able to kill other infected cells (10). Due to this cytotoxic-activating ability, Th1 cells protect against cancer but if they are not tightly controlled, carry the risk of attack against self antigens and cells causing autoimmune damage to host tissues (10). The Th1 cells produce interferon-γ (IFN-γ) and IL-2 (17). Th2 cells promote the humoral response by inducing the differentiation of B cells to produce high amounts of antibodies, are highly effective against extracellular pathogens, but carry the risk of allergic disease (10).

The Th2 subset has a cytokine profile of IL-4, IL-5, IL-10, and IL-13 (17). Allergic disease is an exaggerated (hypersensitive) immune response to an inoffensive environmental antigen that causes damage to the individual. A non-allergic individual would not mount an immune response under the same conditions and is said to be tolerant of that antigen. Th2 cells, in response to recognition of the antigen by specific T cell receptors (TCR), produce cytokines that elicit the recruitment of various cells and cause B cells to secrete antigen-specific

immunoglobulin E (IgE –antibody which mediates hypersensitive reactions). IgE binds via its constant region to specific receptors on mast cells. Antigen-induced cross-linking of these bound IgE molecules causes mast cell degranulation triggering allergic symptoms such as watery eyes, runny nose and sneezing.

APCs process exogenous antigen and present it to helper T cells. The Th TCR recognizes peptide displayed by a class II MHC molecule on the APC. Just like an antibody, a TCR is specific for a particular antigenic epitope. The binding affinity of this tertiary complex impacts the T cell response to the presented antigen. Upon T cell binding, the APC produces cytokines to stimulate the T cells through paracrine action to induce T cell cytokine production. The type of cytokine produced by the APC will determine the Th differentiation to either Th1 or Th2. The professional APCs are macrophages, dendritic cells (DC), and B cells. DCs are the most efficient APCs for naïve T cells since they require no prior activation in order to display class II MHC molecules or have co-stimulatory activity. Mature DCs release large amounts of cytokine and chemokines, preferentially IL-12, as do macrophages (2). DC maturation is reinforced during interactions with T cells by membrane and soluble molecules. At the initial T cell-DC intersection, the cytokine microenvironment has a major influence on the helper T cell differentiation to either Th1 or Th2. IL-12 preferentially promotes the development of the Th1 response. Different molecules, such as ATP, inhibit dendritic cell maturation and convert the typically Th1 skewing dendritic cell into a Th2 skewing cell (13).

Animal models provide evidence that early childhood is an extremely formative time for the immune system in terms of the way in which it learns to respond to the environment (19,29). Humans begin life in an in-utero Th2 environment and whether as a consequence of this or not, neonatal T cells initially produce Th2 cytokines (23). Therefore, a key causative factor in atopic

disease may be the efficiency of immune deviation mechanisms to shift the fetal Th2 response towards Th1.

It is likely that both genetics and the type and route of antigen exposure impact the immune response to an antigen. The mechanisms involved in the interplay between Th1 and Th2 are still being investigated but the evidence generated thus far sheds some light onto the exceedingly complex interaction between the different cells and molecules involved in a hypersensitive (allergic) or tolerant response to a given antigen. The greater the mechanistic understanding of the interplay between Th1 and Th2 cells, the better the ability to develop therapeutics to counteract aberrant immune system allergic response to an innocuous foreign antigen.

Environmental impacts on Th2 polarization

Pregnancy has been described as a Th2 phenomenon due to the maternal helper T cell polarization away from a Th1 response during pregnancy (34). The conjectured reason for the altered cytokine profile during pregnancy is to prevent an immune system rejection of the fetus. The infant begins life in a Th2 environment, which impacts the way the neonate initially responds to antigens. Even though the infant immune system is not fully functional, T cell migration from the bone marrow to the thymus begins during the eight or ninth week of gestation such that the infant can respond to allergen exposure early in life (18). In a study using fetal, neonatal, and adult blood samples, two-color flow cytometry was used to analyze how the leukocyte (white blood cell) populations change during maturation (25). A measurement of the ratio of Th cells to cytotoxic T (Tc – T cells which kill other infected cells) cells yielded a value of 2.9 in fetuses and 2.1 in adults (25). Activated Tc cells are a measure of Th1 cells since Th1 derived IL-2 is required for effector cytotoxic T cell function. Assuming that the fetal ratio is indicative of the maternal immune state due to cytokine polarization, these values demonstrate a

possible physiological mechanism to suppress graft versus host disease in the mother, which, if it were to occur, would result in a spontaneous abortion (25). The relative increase of regulatory Th2 cells during pregnancy may indicate that these lymphocytes contribute to a physiological mechanism to maintain the pregnancy, whereas Tc cells may cause pregnancy loss due to local secretion of Th1 type cytokines (25).

In addition to maternal secretion of Th2-type cytokines, there is another possible route for fetal T cell differentiation. In a cohort of infants and their mothers recruited at birth, umbilical cord blood mononuclear cell (CBMC) samples were positively identified to be of fetal rather than maternal origin using microsatellite analysis and then were tested against a set of 28 overlapping peptides from ovalbumin (OVA) (22). Microsatellites are polymorphic DNA sites that contain a sequence of bases repeated many times which can be used to detect genetic differences among individuals. The CBMCs recognized a number of OVA epitopes, suggesting that the fetal immune response is directed at the native OVA protein containing multiple epitopes as opposed to an unrelated antigen sharing a similar epitope (22). One explanation for why a fetus recognizes OVA without ever having ingested a chicken egg is that low levels of antigen leak across the placenta at concentrations favorable to a Th2 response so that the immune system is sensitized to respond to these antigens prior to birth (22). Some refer to this early antigen exposure as the 'priming' hypothesis (6). It is speculated that by some undetermined mechanism, peripheral dendritic cells redirect neonatally primed T cell responses against environmental allergens towards the nonatopic Th1 phenotype (22). However, this capacity is intrinsically low at birth, and the longer it takes for antigen presenting cells (APC) development to occur, specifically dendritic cells, increases the likelihood of permanent Th2 skewing to result in an atopic phenotype (22). Since various molecules, such as OVA, are able to leak across the placental barrier and affect the fetal immune system, the Th2 cytokines that are present in the

mother are likely able to reach the fetus as well. IL-4 is one such Th2 cytokine that, as demonstrated by Yoshimoto (38), Zauny-Amorim (39), and Noben-Trauth (16), controls Th differentiation into the Th2 phenotype. This prenatal exposure to Th2 cytokines might explain the observation that infants initially have a Th2 cytokine profile (23).

The adult-equivalent patterns of immunity characteristics for both food and aeroallergens are imprinted within the first five years of life, demonstrated by the observed cytokine secretion in response to antigen challenge (37). In a study comparing the T cell cytokine responses of infants with that of adults, an adult pattern of antigen response was observed as early as one-and-a-half years of age as evidenced by T cells proliferation in response to house dust mite (HDM) but not to OVA, while cord blood responded to both types of antigens (37). The different immune response is most likely due to the level of allergen exposure. In comparison to relatively low HDM levels in the air, high doses of OVA are ingested in food, which induces T cell depletion or anergy. Anergy is a state of tolerance that develops upon exposure to high quantities of antigen, such that the T cells no longer recognize the antigen as a threat. As infants are exposed to increased amount of solid food, their bodies become tolerant of OVA and other food antigens in the same way as most adults. When the population of infants in this study was divided on the basis of family history of atopy, they displayed a mixed (both Th1- and Th2-type) cytokine response to both classes of allergens (OVA and HDM) characterized by IL-4 and IL-5 and IFN-γ, whereas the nonatopic risk infants only showed a low level IL-5 response to OVA, indicative of either Th2 or Th0 (a helper T cell type that displays neither a Th1 nor Th2 cytokine profile) cells (37). At five years of age, allergic individuals, defined here by skin reactivity to HDM, produced IL-4 and IL-5 in response to antigen whereas nonatopics only displayed IFN-γ (37).

In a similar investigation of cytokine production in response to antigen challenge tested the hypothesis that individuals who develop atopy in infancy and those who do not show opposing in-utero primed Th2 responses to specific allergens (23). Although the two groups of infants (defined by those who developed atopy at two years and those who did not) have similar cytokine profiles at birth, differing patterns of production were observed with age (23). Nonatopic infants showed a significant IFN-γ increase soon after birth and a gradual IL-4 decrease so that by 18 months, the response was barely distinguishable from an unstimulated control (23). In contrast, atopic infants displayed a persistent IL-4 response but no significant IFN-γ (23).

Age	Atopics	Non-atopics
Birth	IL-4, IL-5, IL-6, IL-10	IL-4, IL-5, IL-6, IL-10
18 mos	IL-4	IFN-γ

Table 1. Comparison of cytokine production at various ages by atopics and non-atopics. [Data taken from reference 23.]

The decreased ability for IFN-γ production in atopics was found to be a defect in the T cells as seen by the fact that in response to APC-independent stimuli of CBMC, IFN-γ production was higher in nonatopics (23). Initial T cell priming commonly occurs across the placenta, and the failure of the immune system to deviate away from the initial Th2 response is due to the decreased capacity for IFN-γ production, since IFN-γ is needed to inhibit Th2 proliferation (23). The impaired IFN-γ producing ability in infants is positively correlated with maternal atopic status. This seems to be a further Th2 skewing in atopic mothers due to mechanisms to protection the fetus from the mother's immune system (21).

Another way in which neonatal immune systems can be exposed to potent immunogens is by passage through the underdeveloped intestinal mucosa. In adults this surface is composed of

tightly packed cells to inhibit undigested food molecules from penetrating between the cells. This impermeability takes a few months to develop, and the infant intestinal mucosa is highly permeable to large molecules in the first months of life (3). Molecules that pass across the intestinal barrier can result in antigenic sensitization to that molecule, which is particularly an issue for infants at high atopic risk (predisposition to develop inflammatory disease in response to otherwise innocuous antigen) who have a greater disposition for developing an allergic response. Familial atopy may not be the most important indicator of allergic disease development however. Saarinen *et al* (24) conducted a longitudinal study following children from birth up through the age of 17 to investigate the role of breast milk in correlation to allergy development. Regardless of atopic heredity, as seen in figure 1, those infants with little or no breastfeeding had a significantly greater rate of allergic disease at 17 years of age, suggesting that breastfeeding can protect against atopic disease (24).

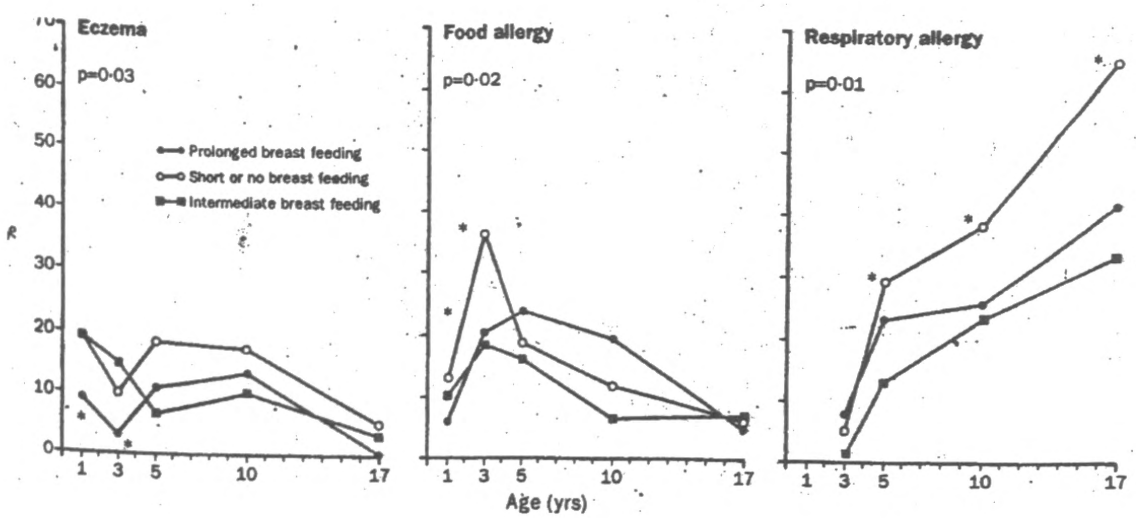

Figure 1. Prevalence of allergic disease (determined by percent of subjects in this study manifesting a particular symptom) in different infant feeding groups as a function of age. Neither eczema nor food allergy is very prevalent in adults. The difference in respiratory allergy between those who received at least some breastfeeding and those who received little or none was statistically significant at ages 3, 5, 10, and 17. (Error bars would be helpful to determine how accurate the differences are in the eczema and food allergy comparisons.) [Figure taken without permission from reference 24.]

Breast milk might promote the maturation of the intestinal mucosal barrier and secretory immune system and reduce the exposure to food allergens by inhibiting their absorption due to the binding of secretory IgA (21). The only problem with this investigation is that it is correlational such that third factors could be the true explanation for the difference. When breast milk is the only source of infant nutrition for the first couple of months, the introduction of solid foods after the neonatal intestinal mucosa develops decreases the likelihood of sensitization to large food allergens (3). Breast milk is the best source of nutrients for the infant and has been feeding infants since mammals first began walking on this earth. It likely has unknown benefits unable to be replicated by formula.

In addition to the rise in the use of infant formula are a number of other changes classified under the heading of "westernization." This is the term given to those characteristics that tend to define the difference between the so-called developed and developing countries including an altered diet, declining family size, improved household amenities, prevention of viral infectious diseases, higher standards of personal cleanliness, and increased use of antibiotics. An observation that substantiates this claim is the discovery in 1994 that atopic sensitization was three times as prevalent in the former West Germany as compared to the former East Germany (32). At the time of the study, the Berlin wall had only been down for a few years, and the lifestyle in Eastern Germany was not as affluent as that in Western Germany. The investigators only drew data on those subjects who were from Germany and of German heritage in an attempt to control for confounding variables and did note that pollution exposure between the two regions was similar.

Another change in recent years is the decreased contraction of serious illnesses during the first few years of life. Childhood respiratory infections have the potential to strongly modify the developing immune system to cultivate a Th1 environment (4,26,27). It is likely that a set of

specific infections, which strongly promote Th1 immunity, has the potential to inhibit atopic disorder by repression of Th2 immunity. Viral and bacterial infection could prevent atopy by the activation of Th1-type T cells and the production of IFN-γ. Those children who are the firstborn into a family have been linked to a greater rate of allergic disease development than their siblings (32). This is proposed to be due to the fact that firstborn children are typically not exposed to many infections until kindergarten whereas later born children are exposed prior to school age by their older siblings (32). This is probably only true in cultures in which the toddlers do not attend day care and are not around other disease-harboring children until they reach school age. In a study of Japanese children, a strong inverse association was found between a positive tuberculin response and a range of atopic characteristics including higher serum IgE concentrations and a predilection for Th2 cytokine production (27). This is an important association between those people who exhibit a delayed hypersensitivity response (Th1 skewing) also having a lower incidence of allergic disease. Similarly, investigators in Guinea-Bissau, West Africa looked at the association between measles infection and atopic development and found a negative correlation between measles infection and atopy with the possible explanation that measles infection hampers allergic sensitization (26). Similarly, asthma prone infants with chronic environmental endotoxin (a protein derived from the cell walls of gram-negative bacteria that is a potent inducer of Th1-type cytokines) exposure have a decreased risk of allergen sensitization through an enhancement of Th1 immunity (4). All of these studies implicate an association between pathogen exposure and a lowered Th2 cell response leading to a reduced risk of allergies.

In addition to pathogenic microorganisms, native bacterial colonies can affect the type of immune response. Germ free mice have no indigenous bacterial microflora in the gastrointestinal tract and are resistant to oral tolerance induction in comparison to mice with

intestinal microflora (29). The germ free mice were able to raise a significant Th2-mediated antibody production after high dose OVA oral challenge, while rendering specific pathogen free mice unresponsive (29). Intestinal microflora hardly seem like an important physiological component, but as seen here, the absence of bacterial colonies hinders the immune system's ability to control a hypersensitive response. Antibiotics are used to kill pathogenic bacteria but also have the side effect of destroying the beneficial bacterial colonies. Mice treated with the antibiotic kanamycin had significantly lower total number of lymphocytes in spleen and Peyer's patches than control mice that did not receive antibiotic treatment (19). Kanamycin-treated mice had higher IL-4 concentrations and lower IFN-γ concentrations compared to controls (19). This Th2 skewing effect was only observed when infant and not adult mice were treated with antibiotics (19) demonstrating that the developing immune system requires the presence of intestinal bacteria to develop a healthy response to benign allergens.

Much of this discovery about which antigens are harmful and which are inoffensive takes place very early in life. The body has developed many ways of dealing with the presence of environmental antigens such that a hypersensitive, allergic response does not develop, but there are certain environmental conditions that are beneficial to direct the immune system towards a Th1 response. The earlier that deviation from a Th2 polarization occurs, the better the probability that an allergic phenotype will not arise. More research is needed to discern the interactions of all of the important environmental and genetic factors in allergic disease development.

Molecular mechanisms

Regardless of how the combined interactions of environment and genetics generate an allergic phenotype, the mechanistic relationship between Th2 and Th1 cells is the same. The complexity of the immune system is illustrated by the large number of molecules and cells

involved in the interaction of these two small cell subsets and researchers are far from understanding all of the intricacies. Important aspects of this mechanism involve the type of cytokine environment present when the cell differentiates, the type of APC displaying the peptide to the TCR, the antagonism between Th1 and Th2, and the affinity of the TCR for the MHC-peptide complex.

The initial IL-4 source to control the differentiation into Th2 has remained elusive although a number of different hypotheses have been proposed which have recently been substantiated by research (14,16,38,39). A potential source of IL-4 is a set of natural killer (NK) T cells from the spleen (38). NK T cells are a subset of $CD4^+$ T cells (CD4 is a cell surface molecule that typically designates Th cells), expressing a limited set of αβ T cell receptors specific for the MHC class I-like molecule CD1. (The CD1 family of molecules associates with β_2-microglobulin and has general structural similarity to class I MHC molecules.) A study to determine the cytokine production of these cells compared the IgE production of normal and β_2-microglobulin deficient ($\beta_2M^{-/-}$) mice (38). If the IL-4 that is produced by NK T cells is important for Th2 commitment, the $\beta_2M^{-/-}$ mice should be IgE deficient since β_2M is necessary for a functional CD1 molecule (38). The logic of this is that β_2-M is necessary for CD1 expression, and NK T cells require antigen presentation via CD1. If β_2-M is not available, CD1 will not be expressed, will not present antigen to NK T cells, and, hence, no activated NK T cells will exist. If NK T cells cannot become activated, they will not produce IL-4. If the NK T cells produce the only source of IL-4 for Th2 cells, Th2 cells in a β_2-$M^{-/-}$ organism will not differentiate and thus cannot produce IL-4 themselves in order to cause B cells to class switch to produce IgE. Normal mice treated with anti-IL-4 have inhibited IgE production (38). $\beta_2M^{-/-}$ mice made minimal or no IgE when challenged with anti-IgD, which usually strongly induces IgE production (38). These mutant mice also failed to secrete IL-4 spontaneously five days after

anti-IgE treatment, whereas the normal mice did (38). However, if IL-4 is in the environment shortly after injection with anti-IgD, the $\beta_2M^{-/-}$ mice can produce IL-4 themselves (38). These results provide strong evidence that the early IL-4 production by NK T cells is essential for IgE production in some situations (38). Similar results were found by Magnan *et al* (14) indicating that the number of NK T cells correlated with the serum level of IgE and IL-4; atopic adults had significantly higher levels of both of these molecules than controls, suggesting a role of NK T cells in Th2 commitment.

An alternative source of Th2 cytokines is the proposed involvement of a subset of γδ T cells in the development of a Th2 response (39). Using TCR δ chain knockout mice (KO), $\gamma\delta^{-/-}$ were created and compared with wild type (39). Repeated intranasal OVA immunizations resulted in IL-5 production in the wild type mice but not in the KO mice who also showed decreased IgE and IgG1 expression (39). When injected with a complex of active IL-4 and IL-4 monoclonal antibody (mAb - in order to increase the stability of IL-4), the KO mice had IgE and IgG1 levels comparable to the $\gamma\delta^{+/+}$ mice (39). Therefore, it seems that γδ T cells are essential for the initial IL-4 production, early IgE and IgG1 synthesis, and the development of the Th2 response in the airways (39). These investigations provide compelling evidence for at least two cell types to provide the initial IL-4 to activate Th2 cells.

A further source of IL-4, although it seems somewhat paradoxical, could be from the naïve helper T cells themselves. In response to previous studies in which possibly interfering IL-4 sources confounded the conclusions, Noben-Trauth *et al* (16) used a very thoughtful design to isolate whether conventional naïve $CD4^+$ T cells are the source of IL-4 (16). TCR transgenic mice with a severe cominbed immunodeficiency (*scid*) background were crossed with IL-4 receptor deficient ($IL\text{-}4R\alpha^{-/-}$) mice (16). *Scid* is a condition in which an organism lacks both B and T cells. However, in this case, the mice are also TCR transgenic such that they have a

functional OVA-specific TCR gene allowing the production of T cells but only of the Th cell type. The *scid* background was used to eliminate NK T and γδ cells as the source of IL-4 since these cells are absent in the particular strain of mice used (16). The APC populations were obtained from irradiated T cell-depleted spleen cells from double deficient mice who can neither produce nor respond to IL-4 (16). Lymph node (LN) cells taken from IL-4Rα$^{-/-}$ mice and cultured with OVA and APC produced detectable IL-4 levels but IL-4Rα$^{+/-}$ did not (16). The addition of anti-IL-4 receptor monoclonal antibody (anti-IL-4Rα mAb) to the IL-4Rα$^{+/-}$ cells resulted in detectable IL-4 levels, suggesting that CD4^{+} T cells rapidly consume IL-4 produced early (16).

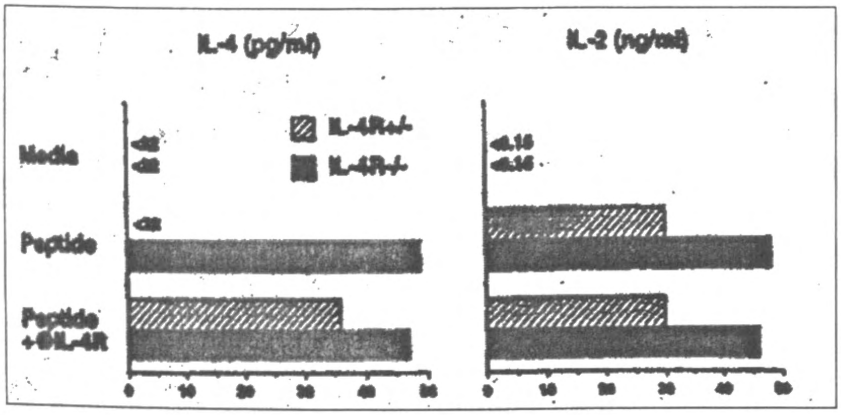

Figure 2. A comparison of the cytokines produced by IL-4Rα$^{+/-}$ (cross-hatched) and IL-4Rα$^{-/-}$ (solid) mice. Upon incubation with peptide (OVA) and APC IL-4 was detected from IL-4Rα$^{-/-}$ but not IL-4Rα$^{+/-}$ mice. The addition of anti-IL-4Rα mAb, both types of mice produced IL-4. IL-2 production was used as a control. [Figure taken without permission from reference 11.]

This study provides persuasive evidence that Th cells can play a part in their own differentiation via autocrine cytokine action. Th cells are a good candidate for providing the initial IL-4 signal because they are at "the right place a the right time." These studies indicate three possible sources of initial IL-4 but there are undoubtedly more. Identifying the initial source of IL-4 is the key to developing therapies to intervene in the earliest phase of Th2 polarization.

As mentioned previously, the cytokine profile of Th1 and Th2 cells are non-overlapping. The presence of IL-4, as demonstrated in the investigations by Yoshimoto (38), Zauny-Amorim

(39), and Noben-Trauth (16), causes differentiation towards the Th2 profile and inhibits Th1 development. IFN-γ and IL-12 are involved in the differentiation of Th1 while inhibiting Th2 development. Atopic individuals typically have a decreased capacity to secrete IFN-γ such that they cannot restrain rampant Th2 proliferation (23).

The proposed mechanism to account for the antagonism between Th1 and Th2 cells was clearly elucidated by an investigation into the effects of certain gangliosides on the production of Th1 and Th2 cytokines (11). Gangliosides are sialic acid-containing glycolipids, which are constituents of various cell plasma membranes. Exogenous gangliosides modulate transmembrane signaling pathways by interacting with the plasma membrane, especially through cyclic AMP (cAMP) related pathways. Culturing T cells with phytohemagglutinin (PHA), a potent mitogen used to stimulate cell proliferation, in the added presence of certain gangliosides enhanced IL-2 and IFN-γ secretion while inhibiting IL-4 and IL-5 production, maximized at 100 nM (11). Plasmids containing the chloramphenicol acetyltransferase (CAT) reporter gene controlled by the IL-4, IL-5, IL-2, and IFN-γ promoters was used to investigate ganglioside control at the transcriptional level (11). The presence of gangliosides in solution enhanced Th1 cytokine (IL-2 and IFN-γ) promoter activities while inhibiting Th2 cytokine (IL-4 and IL-5) promoter activities as evidenced by the presence or absence of the CAT reporter gene products (11). This suggests that these gangliosides regulate cytokine production at the transcriptional level (11). It was also demonstrated that PHA increases cAMP levels and this increase was blocked by the addition of the gangliosides (11). This information indicates that cAMP suppresses Th1 cytokine production (11). When the gangliosides were added directly to the reaction mixture, adenylyl cyclase activity was decreased, with optimal ganglioside concentration being 100 nM (11). This is the same ganglioside concentration needed to generate an optimal Th1 or Th2 response, which is a strong correlation to suggest that the gangliosides

used in this study may act directly on adenylyl cylcase associated with the T cell plasma membrane to prevent cAMP inhibition of Th1 and stimulation of Th2 (11). Differential cytokine production of Th1 and Th2 cells is controlled at the transcriptional level as a result of an extracellular signal working through the adenylyl cyclase/cAMP pathway.

The integral role of cytokines in the differentiation of Th1 and Th2 cells and in allergies in general has underscored the possibility of their use in the treatment of related diseases. The potential problems with this approach are that cytokines have pleitropic effects and typically exert their influence in an autocrine or paracrine fashion, neither of which is will suited to a systemic administration of a particular cytokine. Negative side effects have resulted due to systemic cytokine administration. A recent study was conducted with the administration of IL-18 into the lungs of mice to test the hypothesis that the direct administration of this cytokine could reduce or inhibit a Th2 dominated immune response (33). In order to express IL-18 in vivo, a non-replicating adenovirus encoding the open reading frame of IL-18 was constructed (IL-18:Adv) (33). Airway hyperreactivity (AHR) was induced in mice upon the administration of OVA, however mice also given IL-18:Adv intranasally showed decreased IL-4, IL-5 and IL-13 production and increased IL-12 production, indicating a skewing of helper T cells away from the Th2 subset (33). IL-18 is dependent on IL-12 and IFN-γ to reduce AHR as seen by the fact that treatment with anti-IFN-γ or IL-12 mAbs diminished IL-4 production and reversed the inhibition of AHR conferred by IL-18:Adv (33). An examination of the lungs of mice with established AHR reversed by IL-18:Adv showed greatly reduced airway inflammation, mucus production, and eosinopilia (33). It appears that the administration of IL-18 into the lungs with an adenovirus construct can effectively down regulate a Th2-modulated inflammatory response, which leads to the speculation that IL-18 administration in conjunction with IL-12 acts as an

adjuvant (agent that augments or facilitates an immune response) to enhance the efficiency of allergen based immunotherapies to reverse the asthmatic and allergic response (33).

Although the current prevailing hypothesis is that the Th1- and Th2-type cytokines are antagonistic and that cytokine production is specific to each Th cell subset, some recent work has called into question the validity of this theory in all situations (17). In an investigation to determine the conditions under which IFN-γ inhibits Th2 proliferation, the same Th2 clone may or may not be affected by IFN-γ depending on how the antigen is presented (17). Although not absolutely necessary, IL-1 acts as a co-stimulator for IL-4-induced Th2 activation (17). Clonal proliferation of Th2 cells in the presence of IL-1 was inhibited by the presence of IFN-γ, and the degree of inhibition was directly proportional to the IL-1 concentration (17). However, IL-4 reversed IFN-γ-induced inhibition of the Th2 clone proliferation (17). It appears that IFN-γ blocks Th2 proliferation through IL-1 (17). Plausible mechanisms include the possibility that IFN-γ down regulates the IL-1R expression on Th2 cells or IFN-γ interferes with the IL-1 signally pathway (17). Since B cells do not produce IL-1, either hypothesis is substantiated by the fact that IFN-γ does not inhibit Th cell proliferation when B cells act as APC (figure 3, 17).

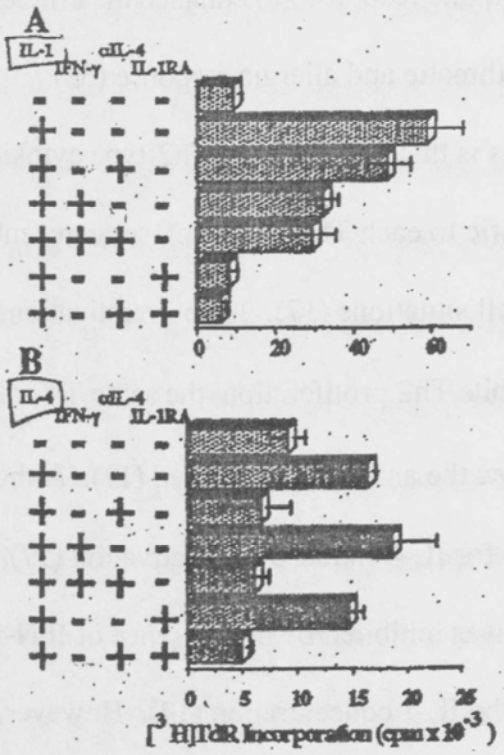

Figure 3. This demonstrates IFN-γ inhibition of IL-1 but not IL-4 associated Th2 proliferation. (A) The presence of IL-1 results in substantial Th2 proliferation as demonstrated by [^{3}H]thymine incorporation, but the presence of IFN-γ decreases that proliferation by half. (B) The presence of IL-4 causes Th2 proliferation, which is not affected by the presence of IFN-γ. [Figure taken without permission from reference 17.]

More than just the presence or absence of a particular cytokine is necessary to predict how a T cell will respond to an antigen; the type of APC has a large impact on activation or inhibition. This study demonstrates how Th2 cells can be stimulated by at least two different mechanisms dependent on the type of cell presenting the antigen.

Nucleotides have been discovered as important immunoregulators and have been shown to affect the functions of B cells, T cells, macrophages through certain cell receptors (1). La Sala *et al* (13) demonstrated that ATP affects DCs as well. The presence of ATP during DC incubation did not affect the mature DC's production of IL-13 or IL-1 but did inhibit IL-12 and proinflammatory (Th1-type) cytokine production (13). T cells primed with ATP-matured dendritic cells displayed a reduced IFN-γ production level and higher IL-4, IL-5 and IL-10 levels compared with T cells primed by dendritic cells without prior ATP exposure (13). The addition

of IL-12 at the time of T cell priming restores Th1 differentiation while inhibiting the release of Th2-type cytokines, indicating that ATP suppresses Th1 differentiation through the inhibition of IL-12 production (13). Extracellular ATP concentrations can increase at the site of tissue injury or inflammation due to traumatic cell lysis or passive leakage from damaged cells (13).

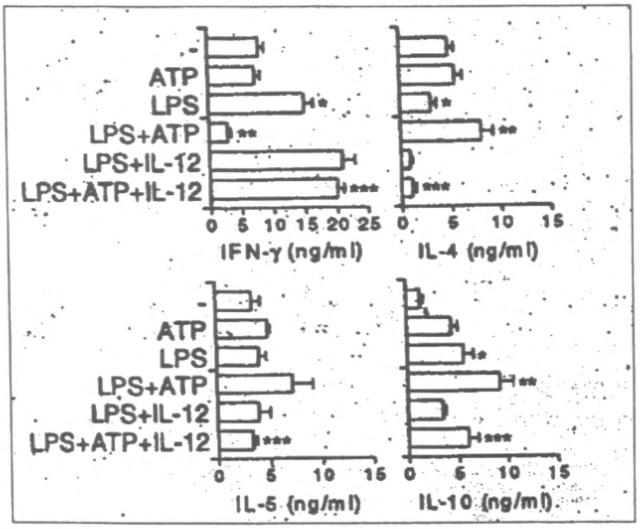

Figure 4. DCs matured in the presence of extracellular ATP have an impaired ability to stimulate Th1-type cytokine production as seen by a decreased IFN-γ production. The presence of IL-12 restores IFN-γ producing ability but inhibits Th2-type cytokine production. [Figure taken without permission from reference 13.]

The mechanism whereby ATP mediates these effects may be very similar to that controlled by cAMP levels as seen in the work by Kanda *et al* (11). At the site of tissue injury or inflammation, ATP might stimulate Th2 polarization to decrease the release of detrimental Th1-type cytokines (13). In some instances, this differential DC maturation might be beneficial, but in the case of chronic inflammation as the result of allergic disease, these APCs might be a partial cause of the characteristic Th2 response to allergens (13).

A Th2 response makes one more susceptible to other environmental antigens since the body is, in a sense, primed to react in that manner. The entire panoply of cells and molecules in the environment affects the type of response generated as a result of antigen exposure. It appears that differentiated Th2 cells are not constrained to retain that phenotype but can be converted

into a Th1 phenotype under appropriate environmental conditions. Restimulating previously polarized Th2 cells either with B cells in the added presence of IL-12 or with DCs shifted the cytokine profile of the Th2 cells to Th1 as indicated by IFN-γ production (10).

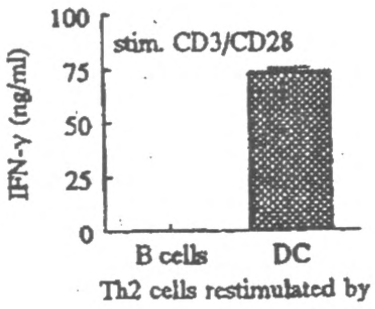

Figure 5. Th2 polarized cells were either restimulated with B cells or DCs as APCs. DCs led the Th2 cells towards a Th1 phenotype, whereas B cells maintained Th2 polarization. [Figure taken without permission from reference 10.]

Even Th2 cells incapable of IFN-γ production from an atopic individual could be inducers of IL-12 production when stimulated with DCs as the APC. It has been assumed previously that IL-12 production is dependent on high IFN-γ and low IL-4 concentrations, but this no longer appears to be true (10). Th2 cells use IL-4 in a co-stimulatory mechanism different from the IFN-γ-dependent mechanism employed by Th1 cells to produce IL-12 (10). The interaction of CD40 and CD40 ligand (membrane molecules on the surface of APCs and T cells respectively) provides the positive signal (secondary activation signal) leading to Il-12 production, which IL-4 upregulates. Yet when LPS induces IL-12 production, IL-4 has an inhibitory effect on IL-12 production (10). The interaction of all molecules and cells in the immediate environment will affect the helper T cell phenotype. The effector cells remain flexible so that, depending on the needs of a particular location, different APCs can be used to control how a particular Th cell responds.

One of the most important components of the interaction between the APC and T cell is the TCR-MHC/peptide complex. A common way to investigate the strength of this interaction

and the impact that it has on the T cell response is to use antigen analogs. An analog was constructed that consisted of alanine substitutions at two different residues of the immunodominant HDM epitope (31). T cells clones, all recognizing the same epitope, were collected from an HDM-allergic individual (31). In response to exposure of a HDM peptide analog, a shift in effector function was observed from a Th2 to a Th1 response as monitored by a rise in IFN-γ production (31). The analog downregulated CD3 (a membrane polypeptide complex associated with the TCR, functioning in delivery of the TCR membrane signal to the nucleus) cell surface expression compared to the wild type HDM antigen, implying that the analog may have a higher TCR affinity since CD3 down-regulation is a direct measure of TCR occupancy (30). The binding of a TCR with a MHC/peptide complex results in the down-regulation of nonengaged receptors. Tight binding will decrease the need for the involvement of other TCRs to activate the cell. Another reflection of tighter TCR-analog binding was the observation that this complex induced more anergy than the native HDM at all doses tested (31). Since the analog stimulates an increased production of IFN-γ, which is paralleled by CD3 down-regulation, a different threshold of signaling arising from changes in TCR ligand affinity is implicated (31). The surface expression of a few less CD3 complexes will not effect anergy induction so the stronger TCR binding must influence receptor signaling in some fashion that has yet to be determined. Anergy induction is important in this discussion because anergy reflects a state of unresponsiveness to a given antigen. If an individual does not respond to an antigen then they cannot mount a hyperactive immune response.

Classical immunotherapy makes use of the anergy concept. Injecting increasing doses of allergen extracts causes the body to recognize the antigen as innocuous by inducing a state of antigen tolerance. However, this therapy can induce severe systemic reactions due to cross-linking of allergen-specific IgE on mast cells and has the potential to activate Th2-polarized cells

leading to an unfavorable hypersensitive reaction. The use of synthetic immunodominant epitope peptides (such as the analog constructed in the above study by Verhoef *et al* (31)) have been used to circumvent these side effects since the analogs are only a short peptide sequence incapable of crosslinking IgE molecules. These analogs might mediate their effects through modifications of TCR-contact-residues that affect the early biochemical events leading to T cell activation by changing the dissociation constant of the TCR from the MHC-peptide complex (9). Kinetic studies demonstrate a relationship of T cell activation to trimolecular binding affinity of the TCR-peptide-MHC complex (8). A prolonged TCR interaction with peptide/MHC complex leads to a Th1 response, apoptosis, and anergy, whereas a low affinity trimolecular interaction leads to a Th2 response (8)

In an investigation of the modulation of a Th2 response based on these theories, twelve peptide analogs were synthesized based on single alanine substitutions to the immunodominant $OVA_{323-339}$ epitope (9). Of these, analog 336E-A bound with the same affinity to the MHC class II molecule as the wild type immunodominant epitope but was a more potent inducer of naïve T cell proliferation and caused a cytokine shift from Th2- to Th1-type cytokine production (9). This analog also ameliorated an ongoing in vitro Th2 response to $OVA_{323-339}$ and decreased eosinophil infiltration in mice with chronic asthma (9). Due to these apparent shifts from a Th2 to a Th1 response, it appears that 336E-A binds with higher affinity to the $OVA_{323-339}$ –specific TCR than the wild type peptide (8,9) which probably accounts for the differential response (8). Subcutaneous native OVA or 336E-A analog administration resulted in a strong, proliferative immune response and an increase in the number of T cells draining into brachial LN (the lymph nodes that drain from subcutaneous antigen injection), whereas the use of the immunodominant $OVA_{323-339}$ epitope resulted a weak response (8). Even though 336E-A induced IFN-γ production in vitro, the lung draining LN cells from the asthmatic mice after 336E-A

administration did not show IFN-γ production (9). A possible reason for this is that Th2 cells became anergic and stop producing the Th2 cytokines needed to maintain the airway hyperresponsiveness but did not actually switch to a Th1 phenotype (9). High antigen concentrations lead to anergy, which correlates well with the observation that high affinity TCR-MHC/peptide interactions direct towards anergy due to overstimulation of the Th2 cells (8). Optimizing the binding strength of the TCR to the MHC/peptide complex will maximize the beneficial immune polarization away from the asthmatic phenotype (8).

Beyond the implications of this research for immunotherapy is the fact that these mechanisms most likely operate in vivo in response to a given antigen. Those people who develop an allergic response characterized by a Th2 polarization might have TCRs that bind with a reduced affinity to that particular antigen, whereas antigens against which one does not mount an allergic response are recognized by TCRs that bind with a higher affinity. This seems like a plausible explanation to explain how different people respond to antigens differently. It would be interesting to investigate whether the strength of this interaction could be one determining factor in controlling the differentiation of a helper T cell into a particular subset. The mechanism of TCR diversity offers an explanation for the random chance event of one person having a TCR with a strong MHC/peptide affinity and another person having low TCR affinity. TCRs are generated in a similar fashion to immunoglobulins in order to generate a large repertoire of receptors able recognize the presence of any potential antigen. Human DNA contains multiple gene segments for the different regions of the TCR. During T cell maturation, these segments rearrange in seemingly random ways with the addition of extra nucleotides during segment joining to generate as many as 10^{13} possible amino acid sequences (5). There might be other mechanisms yet to be discovered that are less random chance gene arrangement events that modulate TCR binding strength. The TCR-MHC/peptide binding affinity is one of the most

basic events controlling how cells respond to a given antigen. Being able to modulate the immune response at this level would appear to hold high therapeutic potential to help those allergic individuals who have T cells that bind with low affinity to a native antigen.

Conclusion

The immune system is an exceedingly intricate interaction of many different molecules and cells. The high amount of complexity increases the possibility of some component going awry, but even so, most of the time everything runs smoothly. It takes something to go amiss in order to highlight the importance of a particular component. An investigation into the relationship between Th1 and Th2 cells emphasizes many possible ways in which the immune system can fail to react to an antigen 'properly.'

The TH1/Th2 cell interaction indicates the importance of every piece of the mechanistic puzzle, from the omnipresent cytokines, to the differing APCs, to the interaction of the TCR-MHC/peptide complex. All pieces are important and have implications for every other part. If the TCR-MHC/complex binds with low affinity, Th2 cytokines will be produced. If a B cell is the APC, it will not be affected by the presence of IFN-γ to limit Th2 polarization. These are just two scenarios that could have a part in the development of a hypersensitive response, but there is still so much that has yet to be discovered. So much is unknown with regard to how all of the different cytokines produce their effects and what role environmental factors contribute to the development of an allergic phenotype.

Since it appears that humans enter the world predestined, in a sense, to react to any incoming antigen via a Th2 response, the body must have an intrinsic mechanism to deviate from this response towards Th1. Both genetics and the environment play a part in whether one accomplishes a successful Th1 conversion. If in fact the incidence of allergic disease is increasing around the world, an understanding of how all of the different components fit together

is even more important in order to determine what factors are contributing to the increase. Humans have substantially altered the environment such that it is not surprising that environmental factors are important. If the environment cannot be altered back to the way it was in the past, either because it is impossible or undesirable, then therapeutic controls can be developed to target those specific areas of the Th1/Th2 mechanistic balance that are in error. It will take an appreciation of the entire picture encompassing the human immune system and the environment in order to target one type of protein in one cell type, quite possibly in a single location.

Increasingly it seems that people are realizing how harmful the hypersensitive immune response can be in some situations, such as the national coverage of the recent death of a child in Spokane due to a severe peanut allergy. Nevertheless, a heightened international awareness of the problems associated with the allergic state is needed since disparities of allergic incidence appear to exist within and between countries. An increased consciousness would be beneficial especially when effective, safe immunotherapies are available for distribution to combat this disorder so that allergic individuals can receive necessary aid.

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